ISSN 2347-4289

Detection Of Chromosomal Instability And Subtelomeric Rearrangements In Sick Neonate And Children With Multiple Congenital Malformations.

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ABSTRACT: Purpose-In order to assess major chromosomal abnormalities among sick neonate with dysmorphic feature and delayed milestones in Kolkata, a chromosome aberration survey was initiated in collaboration with Dr. B. C. Roy Post Graduate Institute for Pediatric Science (Kolkata) is in progress. **Methods**- In last two years, we have screened about 120 sick neonates (Indicated cases as per clinical findings). Cytogenetic analysis of blood lymphocytes were studied with High Resolution GTG-banding analysis by using Chromosome profiling (Cyto-vision software 3.6) on their chromosomes. **Results**- The result shows that among 120 patients 22 cases have chromosomal abnormality (2% cases) and 36% (8 cases out of 22) have chromosomal structural variation in sick neonate with gross dysmorphic features with MR which is correlated with International Data. **Conclusions**- Present data shows 1% (total 22 cases) was affected with chromosomal anomalies represent about 1800 sick neonate screening in West Bengal Population in last two years. This report provides valuable addition to the growing literature in Birth Defects Database in India.

KEYWORDS: Chromosomal abnormality, Sick Neonate, Mental Retardation, Dysmorphic features

1. INTRODUCTION

Malformations are a major cause of morbidity and mortality in full term infants and genomic imbalances are a significant component of their etiology. However, the causes of defects in many patients with multiple congenital malformations remain unexplained despite through clinical examination and laboratory investigations give rise to the frequency of Chromosomal aberration [1]. Chromosomal abnormalities are the major cause of congenital abnormalities in human genetic diseases, associated with, mental retardation, dysmorphic features, developmental delays, as well as multiple congenital anomalies [2], [3]. The most common chromosome abnormalities in newborns are trisomy 21 and sex chromosome abnormalities [4]. To achieve optimal management and treatment for these patients the early diagnosis of these chromosomal disorders is very much important. So, standard chromosome analysis by karyotyping remains the first line test in routine diagnostic clinics .It is observed that in many pediatric wards in India, newborns with dysmorphic features and multiple congenital abnormalities sometimes are not genetically evaluated due to lack of Gene Testing facilities or Genetic counseling Clinic .It is a known fact that each chromosome have thousands of genes and it is not surprising that chromosomal disorders with clinically altered phenotype have large to minute structural chromosomal rearrangements which can be determined by the standard cytogenetic method. It is also difficult to determine the cognitive impairment in neonatal period. In this scenario our main objective was to determine the unbalanced structural chromosomal abnormality such as partial chromosomal deletion or duplication or unbalanced rearrangements like partial trisomy or partial monosomy or unbalanced tranlocation. Though loss and gain analysis by chromosome profiling by Cytovision 3.6 software is an easy and cheap tool for diagnosis before genetic counseling, Standard chromosome analysis and chromosome profiling sometimes prove unrevealed, and still many cases remain as such where the patients left without diagnosis. Patients with atypical phenotypes for a particular syndrome or suspected for a syndromic case by the clinicians can proceed for diagnosis by florescence in situ hybridization or FISH and DNA based mutation analysis techniques (Array CGH) for further confirmation. In this study, we investigate 120 cases with multiple congenital abnormality, dysmorphic feature and mental retardation where 22 cases are identified as abnormal chromosomal constituent. Among these most of the cases are numerical chromosomal abnormalities like trisomies and presence of mosaic cell lines in autosomes as well as sex chromosome, about 8 cases, we found partial chromosomal deletion or duplication or unbalanced rearrangements like partial trisomy or partial monosomy or unbalanced translocation. We have performed FISH in a single case to determine the deletion in chromosome no 22 and hematological and DNA based mutation study in two suspected thalassaemic cases. Present survey for chromosomal aberration among of sick neonates and children with dysmorphic features (Hospital record shows 75 unselected cases per month admitted in neonatal and pediatric intensive care unit or NICU & PICU) seems to be a significant data from India and this report provides additional information to the growing literature in Birth Defects Database.

2. MATERIALS AND METHODS

2.1Sample Collection

The project proposal was approved by the Institute Ethical committee of Dr. B. C. Roy Post Graduate Institute of Pediatric Sciences, Kolkata and allowed us to collect all the Specimens from Neonatology and Pediatric Ward along with patient data sheet contain all basic clinical information and investigation reports assigned by the attending consultant pediatricians as per our guide lines (printed form contains all the information about clinical features of sick neonates).

2.2 Cytogenetic Study

The collected blood samples were taken to the cytogenetic laboratory of Institute of Genetic Medicine and Genomic Sciences. The Cells (Peripheral Blood) were cultured in culture media. The culture was then processed using standard protocol of leukocyte culture [5]. After processing of the culture, metaphases plate were prepared and subjected to G-T-G Banding technique [6] for analysis of the chromosome abnormalities, 20 metaphases plates were then analyzed.

2.3 Chromosome Profiling

To evaluate structural variation (Loss or Gain analysis) in all routine GTB Chromosome, profiling of chromosomes were done by using automated karyotype system-cytovision version 3.6 Applied Imaging.

2.4 FISH Analysis

FISH analysis was done by Vysis LSI DiGeorge/VCFS Region Probe Dual Colour- a two colour probe mixture that contains the spectrum orange LSI TUPLE 1(HIRA) probe and the spectrum green LSI ARSA (arylsulfatase A gene) which maps very close to the chromosome 22 telomere. This probe designed to detect deletion of the TUPLE 1 region of chromosome 22. The spectrum green LSI ARSA serves as a control for Chromosome 22 [7].

2.5 Molecular Analysis

The two suspected thallasaemic patients were evaluated for Haemoglobin, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), R.B.C.s, Red Cell Distribution Width (Rdw), Haematocrit (Hct) etc. by Automated Cell Counter (Medionic, Mark). The complete and final screening was done through Hb-variant analysis by High Performance Liquid Chromatography (HPLC). Hb variants (HbA, HbF and HbA2/E) were estimated by HPLC (Variant I, Bio-Rad, USA) using manufacturer's protocol. On the other hand, DNA was isolated from white blood cells, using a DNA isolation kit for mammalian blood (Qiagen). Patients were screened for common β-thalassemia mutations of Eastern India like IVS 1-5(G-T), IVS1-5(G-C), codon 8/9 (+G), codon 26 (G-A), and Fr. 41/42 (−TCTT) along with Cod 26 (G-A) for HbE. The screening was performed by PCR based technique, Amplification Refractory Mutation System (ARMS) as described by Old et al. [8].

3. RESULTS

The result shows that among 120 indicated cases (patient with dysmorphic features associated with congenital defects) 22 cases have chromosomal abnormality (14 cases have numerical defects and 8 cases with structural chromosomal instabilities). In numerical chromosomal abnormality 4 cases showed Down syndrome (Female=2 cases with karyotype 47XX,+21 and male=2 with karyotype 47XY,+21) , 2 cases are mosaic Down syndrome and single case of male with karyotype 46XY/47XY,+21) or Down Syndrome variant with different degree of mosaicism, 2 are trisomy 18 or Edward syndrome (single case of male with karyotype 47XX,+18 and single case of female with karyotype 47XY,+18), 3 cases having trisomy 13 or Patau syndrome (single case of female with karyotype 47XX,+13 and two cases of male with karyotype 47XY,+13) were also detected. A single case of Tuner Syndrome (45;XO), 2 cases of mosaic Tuner Syndrome (46XX/45XO) or Turner Syndrome Variant with variable degree and single case of 46XX/46XY syndrome were also found in the present study (See the Table-I)(Figure-1). Among the 8 cases of structural chromosomal abnormality, one case showed the karyotype 46XY,t(14q:21q)- a case of Down's Syndrome, one have the karyotype 46XY;del(22q)- a case of Di-gorge Syndrome, one have the karyotype 46XY;del(3p26.3), one have the karyotype with 46XY;del(20p) –it is a case of Allagile Syndrome and one have the karyotype with 46XY;der(9) and a single case of 8p deletion syndrome with karyotype 46XY;del(8p) . We also found 1 male and 1 female of 1p36deletion syndrome with the karyotype of 46XY;del(1p36.21) and 46XX;(1p36.3) respectively (See the Observation Table-II)(Figure-2 and Figure-3). During the present study we also found two cases with severe hypodiploidy with the karyotype of 41XY;(-8,-9,-18,-21,-22),del10q, del14q, del16p and the second case 39XY;(-1,-11,-9,-20,-22) and another case of mosaic hyperdiploidy with karyotype of 46;XY/46;XY,(-5,-10,-9,-12,-16)(+5 marker chromosomes). FISH analysis results shows deletion of the 22q11 region containing the TUPLE 1 gene, the spectrum Orange TUPLE 1 probe signal will be absent on chromosome 22 homologue and present on the other chromosome 22 homologue. The Spectrum green LSI ARSA control probe signal will be present on both chromosome 22 homologues (Figure-4). The hematological study in the two suspected thalassaemic cases shows beta-talassaemia trait in one male patients and another female patients have HbE-beta thalassaemia. Mutation analysis study shows that IVS 1-5(G-C)/+ in male and IVS 1-5(G-C)/Codon26 in female patient.

4. DISCUSSION

Routine cytogenetic diagnostic laboratory microscopic chromosome variation usually carried out based on an International System for Human Cytogenetic Nomenclature (ISCN-2005), and are not actually easy to identify. These structural abnormalities usually detected with optical microscopes are aneuploidies, marker chromosome, gross rearrangements and variation in chromosome size. The frequency of these abnormalities in human population is thought to be as 1 every 375 live births by putative information [9]. Some genetic diseases are suspected to be caused by these structural variations, but the relationships are not very certain. It is not plausible to divide these variants into two classes as "normal" or "disease", because the actual output of the same variant will also vary. Structural variations also have its function in population genetics. Different frequency of a same variation can be used as a genetic mark to infer relationship between populations in different areas. In order to understand chromosomal structural variation in our region (West Bengal) this present collaborative project was initiated, in which we have screened chromosome abnormalities among the neonates and children with dysmorphic features associated with congenital defects admitted each day in the hospital. All the patients were clinically examined by attending clinician, registered and selected for present studies. Most of the patients were admitted with severe critical condition and follow up for repeat specimens were failed due to sad demise or referring to other Hospitals for

further managements. In 8 out of 22 patients in this study (see table) the aberration occurred de novo; two of them had complex rearrangements with both a duplication and a deletion . As shown by testing the parental samples, none of the abnormalities detected in this study was derived from a parental balanced translocation. Lacking of familial cases is not in line with the observations in large studies [10], [11] who assessed that 60% and 50% of pathogenic imbalances, respectively, originated from a parent with a balanced translocation. However, paternity was not checked in the present study.

5. CONCLUSION

Human chromosomes harbor hundreds of structural differences including deletions, insertions, duplications, inversions, and translocations. Collectively, these differences are known as "structural variation" (or, "SV") at present Genetic Science. Any two humans differ by thousands of structural variants which vary greatly in size and phenotypic consequence. The present study (screening) result shows presence of structural variation (SV) along with recognized syndromes like Down Syndromes (4) Edwards Syndrome (2) Patau Syndrome (2) Turner Syndrome (2) Sex Chromosomes Abnormality (1) Others (4) and chromosome rearrangements (8). Among them two cases has shown typical clinical Phenotype (Beta-Thalassaemia associated with microcephaly, congenital Cataract and rare cases of microdeletion 1p36) reported probably first time from India [12]. Though any correlation between the thalassaemia and 1p36 deletion could not be established in this present scope, we hope this kind of novel information will be extremely valuable for further in detail study and enrich the growing literature. As a beginner in understanding the role of SV to congenital developmental defects and complex disease, our present preliminary survey data on frequency of chromosome anomalies are also well correlated with other international studies [13], [14]. Present survey for chromosomal aberration among of sick neonates and children with dysmorphic features (Hospital record shows 75 unselected cases per month admitted in neonatal and pediatric intensive care unit or NICU & PICU) seems to be a significant information based report from India which in time will provide a large scope of in depth study and help the Birth Defects Database to enrich further.

Funding statement: "This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All the research work done by the affiliated institutions' funding.

Competing Interests Statement: The authors declare that they have no competing interests.

Contributorship Statement: Dr. Sudipa Chakravarty drafted the manuscript. Dr. Amit Chakravarty helped with the discussion and data summary. Puspal De perform all the experiments, laboratory work and analysis. Dr. T. K. Saha clinically identifies the indicated case. All authors read and approved the final manuscript.

Data Sharing Statement: We cannot share any unpublished data with other laboratory or person.

Patients Consent Statement: The signed consent from all the patients are taken before test was performed and kept them as official documents. In case of any unusual condition it will be presented in front of the concern persons.

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Table-I
Numerical Chromosomal Abnormality

Sl No	Type of Chromosomal Abnormality	Karyotype	Conclusion
1	Numerical	47;XX,(+21)	Down Syndrome
2	Numerical	47XY,+21	Down Syndrome
3	Numerical(Mosaic)	46XY/47XY,(+21)	Down Syndrome Variant
4	Numerical(Mosaic)	46XX/47XX,+21	Down Syndrome Variant
5	Numerical	47XX,+18	Edward Syndrome
6	Numerical	47XY,+18	Edward Syndrome
7	Numerical	47XY,+13	Patau syndrome
8	Numerical	47XX,+13	Patau syndrome
9	Numerical	45;XO	Turner Syndrome
10	Numerical(Mosaic)	46XX/45XO	Turner Syndrome Variant
11	Numerical	46XX/46XY	Sex chromosome abnormality
12	Numerical	41XY;(-8,-9,-18,-21,-22),del10q, del14q, del16p	Severe hypodiploidy
13	Numerical	39;XY(-1,-11,-9,-20,-22	Severe hypodiploidy
14	Numerical(Mosaic)	46;XY/46;XY,(-5,-10,-9,-12,-16)(+5 marker chromosomes).	Severe hypodiploidy with Marker Chromosomes

Table-II
Structural Chromosomal Abnormality

Sl No	Type of Chromosomal Abnormality	Karyotype	Conclusion
1	Structural	46XY,t(14q:21q)	Down Syndrome Variant
2	Structural	46XY;del(22q)	Di-gorge Syndrome
3	Structural	46XY;del(3p26.3)	ALL
4	Structural	46XY;del(20p)	Allagile Syndrome
5	Structural	46XY;der(9)	Derivative 9 syndrome
6	Structural	46XY;del(8p)	8p deletion Syndrome
7	Structural	46XY;del(1p36.21)	1p36 Deletion Syndrome
8	Structural	46XX;(1p36.3)	1p36 Deletion Syndrome

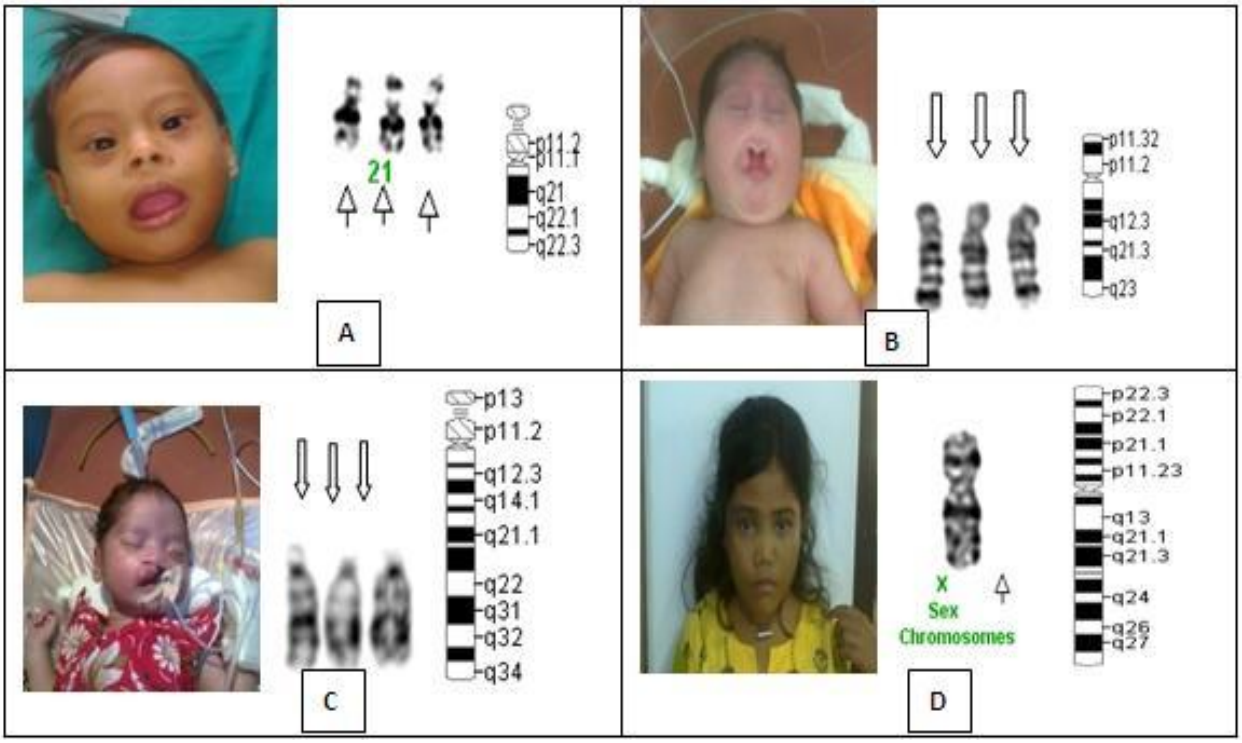

Figure: 1- *Figure Showing Phenotype, Ideogram with band position of the particular chromosome, abnormal chromosome with Numerical Chromosomal Abnormality. A- Down's Syndrome, B- Edward Syndrome, C- Patau Syndrome. D- Turner Syndrome.*

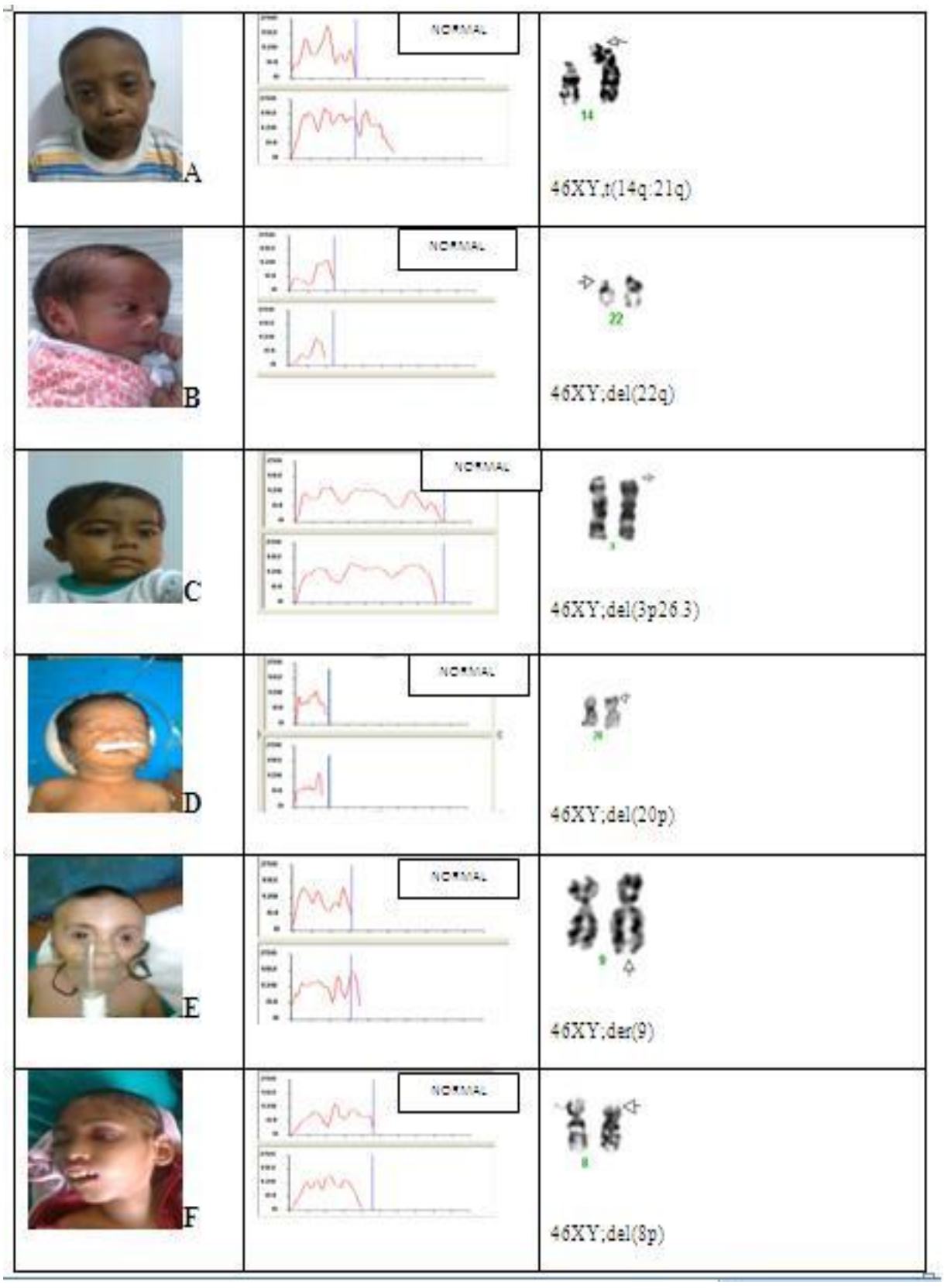

Figure:2- *Figure Showing Phenotype, Ideogram with band position of the particular chromosome, Abnormal chromosome with Structural Chromosomal Abnormality and Chromosome Profile (Loss and Gain Analysis) in contrast of Normal Chromosome. A- Down's Syndrome with Robertsonian Translocation, B- Di-Gorge Syndrome, C- 3p Deletion Syndrome. D- 20p Deletion Syndrome, E- Derivative 9 syndrome, F- 8p Deletion Syndrome.*

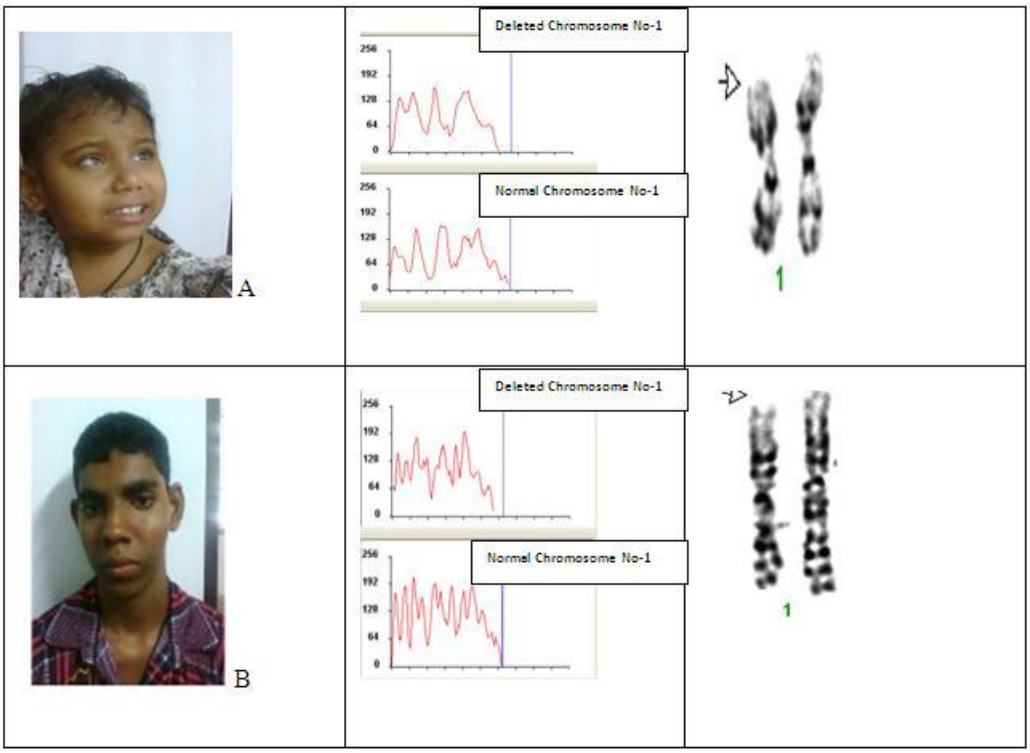

Figure: 3- *Figure showing typical clinical Phenotype in rare cases of microdeletion 1p36. A- Beta-Thalassaemia associated with congenital Cataract, B- Beta-Thalassaemia associated with microcephaly.*

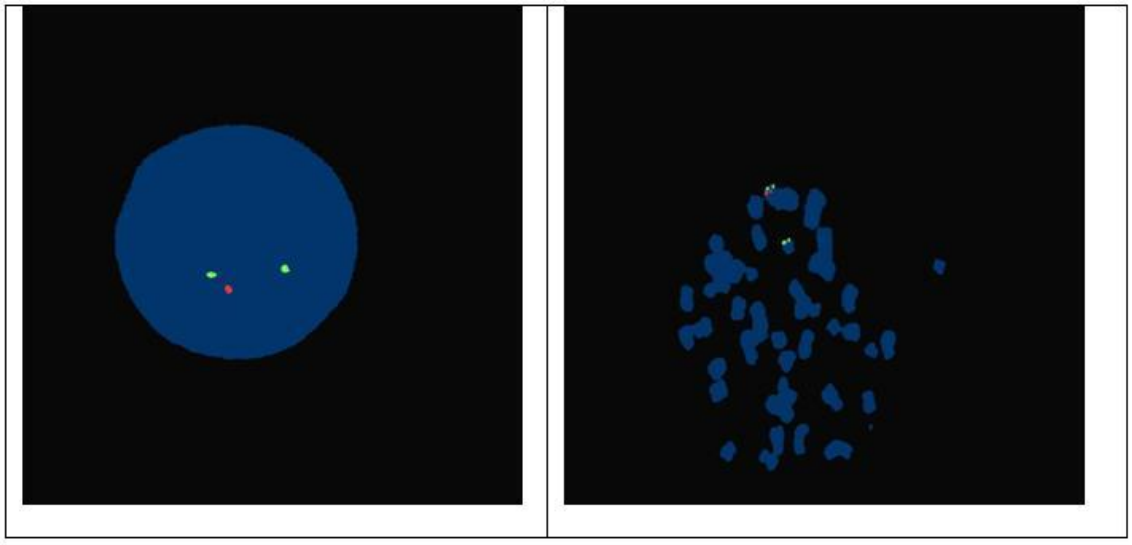

Figure: 4- *FISH analysis results shows deletion of the 22q11 region containing the TUPLE 1 gene, the spectrum Orange TUPLE 1 probe signal will be absent on chromosome 22 homologue and present on the other chromosome 22 homologue. The Spectrum green LSI ARSA control probe signal will be present on both chromosome 22 homologues.*